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(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF ALLERGIC DISORDERS

(57) Abstract: The present invention relates to methods of lowering IgE levels and to methods of treating allergy, by administration of a therapeutically effective amount of live probiotic bacteria, or a live probiotic bacteria-containing composition. The present invention also relates to methods for identifying useful probiotic bacteria and to compositions containing probiotic bacteria.

"COMPOSITIONS AND METHODS FOR TREATMENT OF ALLERGIC DISORDERS"

TECHNICAL FIELD

The invention relates to the field of allergic disease prevention and/or treatment, and in particular to probiotic bacteria which have the capacity to prevent and/or treat allergic disease.

BACKGROUND ART

Allergy is a clinical syndrome affecting about one third of the population, manifest as rhinitis, asthma, eczema or food hypersensitivity. Although target tissue changes play a role in determining the pattern of disease, the central abnormality (known as atopy) is the genetically influenced propensity to develop an IgE antibody response following antigen exposure. Increasingly it is recognised that the characteristics of the gut bacterial flora may drive IgE-promoting immunological mechanisms possibly by affecting the cytokine balance produced by CD4+ T lymphocytes. Modification of the gut flora may modulate the balance of this T cell response, and indeed differences in faecal quantitative bacteriology have been described within populations of children who differ in their incidence of asthma. A means by which to achieve a reduction in the level of IgE is desirable.

The literature that exists describes the use of yoghurt which contains certain potentially beneficial bacteria but the content of such bacteria in yoghurt is relatively low (usually 10^5 to 10^7 organisms) and is largely unknown. Further, many studies show that commercially produced yoghurts contain large numbers of other, not necessarily beneficial bacteria, and often only dead bacteria. Such food products are consumed in an ad hoc and uncontrolled manner. There is no indication or suggestion of how such products, or how much, should be consumed to achieve beneficial effects.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

SUMMARY OF THE INVENTION

It has unexpectedly been found that certain bacterial species when introduced **live** into the digestive system in sufficient quantity have the capacity to downregulate IgE antibody, and thus prevent and/or treat allergic disease. Further, the timing of

administration of the bacteria in relation to antigen/allergen exposure may also be of significance in certain circumstances.

According to a first aspect. the present invention provides a method of lowering IgE levels by administration of a therapeutically effective amount of live probiotic bacteria. or a live probiotic bacteria-containing composition, to a subject in need thereof.

According to a second aspect there is provided a method of prophylactic or therapeutic treatment of allergy by administration to a subject requiring such treatment a therapeutically effective amount of live probiotic bacteria, or a live probiotic bacteria-containing composition..

Preferably, the probiotic bacteria is, or the probiotic bacteria-containing composition includes, *Lactobacillus*.. Most preferably, the *Lactobacillus* is *Lactobacillus acidophilus* and/or *Lactobacillus casei*.

Preferably, IgE is lowered from elevated levels induced by an allergen or as a consequence of an allergic disorder.

Preferably, the probiotic bacteria, or a probiotic bacteria-containing composition, is administered at the time of exposure to an allergen or shortly thereafter.

In the context of the present invention the term "exposure" with reference to an antigen or allergen includes natural exposure such as for example day-to-day contact with or ingestion of food products and the like, or seasonal exposure or re-exposure to allergens such as pollen or other air-borne allergens or by contact with skin and other body surfaces which may involve contact with synthetic materials or natural substances. In these circumstances the probiotic bacteria or compositions containing such bacteria are preferably administered at the time of exposure to the antigen/allergen or shortly thereafter. In seasonal exposure or re-exposure to allergens, the treatment is preferably commenced at the beginning of the season or shortly thereafter. However, the term "exposure" when used with reference to antigen/allergen also includes artificial exposure or administration such as for example the injection of antigen/allergen in desensitisation procedures or under-tongue administration of antigen/allergen. In these circumstances the antigen/allergen is preferably co-administered (co-presented) with the probiotic bacteria or a composition containing them. However, probiotic bacteria may also be administered shortly after administration of the antigen/allergen.

Preferably, the subject in need thereof is selected from the group consisting of high risk infants; those subjected to high risk occupational exposure to allergens; those exposed to high risk allergens; those having recognised allergy to specific allergens; and those prone to anaphylaxis. Preferably, the high risk infants are children of parents who both have allergic disease. Preferably, those subject to high risk occupation exposure to allergens are selected from the group consisting of aluminium smelter workers, woodworkers, chemical factory workers, and those working with latex-containing materials, especially gloves. Preferably, those exposed to high risk allergens are exposed to bee venom. Preferably, exposure to high risk allergens is parenteral exposure. Preferably, in those having recognised allergic disease to specific allergens, the specific allergens are present in the pre-pollen season, foods or latex-containing materials. Preferably, in those prone to anaphylaxis, the trigger for the allergic response is insect envenomation, and food and drug sensitivities.

Preferably, the probiotic bacteria or probiotic bacteria-containing composition is in tablet or capsule form. However, it will be clear to those skilled in the art that the probiotic bacteria may be present in a food source such as a yoghurt or other dairy product.

Preferably the amount of probiotic bacteria administered to a human subject is at least 10^{10} live bacteria. More preferably the amount administered is from about 10^{10} to about 10^{12} live bacteria.

The required dosage amount will vary according to the severity of the allergic condition, the nature of the allergic condition, age of the subject and other standard clinical parameters. These parameters as well as the required dosage can be easily assessed by those skilled in the art.

In human subjects it is preferred that the probiotic bacteria or a composition containing them be administered daily.

According to a third aspect the present invention provides the use of live probiotic bacteria for the manufacture of a medicament for lowering IgE levels.

According to a fourth aspect the present invention provides use of live probiotic bacteria for the manufacture of a medicament for treating allergy.

According to a fourth aspect, the present invention provides a method of identifying a bacterial species capable of lowering IgE levels in a mammal including:

a) administration of the bacterial species to a mammal;

b) administration of an allergen (antigen) to the mammal; and

c) determination of the IgE antibody level in the mammal after treatment with the bacterial species and comparison with a control mammalian which a bacterial species was not administered.

wherein steps (a) and (b) can be performed consecutively in any order or simultaneously.

Preferably, the bacterial species is a *Lactobacillus* species and most preferably it is *Lactobacillus acidophilus* or *Lactobacillus casei*.

The animal model for identifying useful probiotic bacteria preferably makes use of the mouse. However other animal models may be developed on the same principle as disclosed herein.

In the animal model the bacterial species is preferably administered orally however it may also be administered intraperitoneally and other means. Most preferably, the bacterial species is administered in an amount of 10^8 to 10^{11} bacteria, and more preferably, in an amount of 0.6 to 1.0 x 10^{10} bacteria. Preferably, 1 to 20 oral doses are administered prior to administration of the antigen and most preferably 4 to 8 oral doses are administered prior to administration of the antigen. Preferably, administration of the oral doses is at 1 to 5 day intervals and, most preferably, at 2 day intervals. Preferably, administration is over a 1 to 3 week period and, most preferably, over a 1 to 2 week period.

The preferred antigen/allergen used in the animal model is egg albumin (OVA) however it will be clear to those skilled in the art that other antigens can be used as allergens in this model should that be required. Preferably, the antigen is administered at a dose of 4 to 10 μg, and most preferably, at a dose of 8 μg. Preferably, the antigen is administered intraperitoneally. Preferably, the bacterial species is administered at the same time or 1 day after administration of the antigen/allergen. However, in certain embodiments the antigen/allergen can be administered after the bacterial species and in such circumstances the antigen/allergen is administered preferably 1 day after the administration of 4 to 8 oral doses of bacteria.

Preferably, administration of the bacteria species is continued after administration of the antigen. Most preferably, approximately 8 oral doses of bacteria are administered. Preferably, the bacteria are administered at 2 day intervals.

Preferably, the IgE antibody is obtained from serum. Preferably, the serum is collected approximately 14 days after administration of the antigen. Most preferably, the IgE antibody level is determined using an ELISA assay. However, the skilled addressee will recognise that other IgE antibody assays may also be used.

Preferably, the allergic disease is selected from the group consisting of asthma, eczema, hayfever and food allergy.

According to a fifth aspect, the present invention provides a bacterial species identified by the method of the fourth aspect.

According to a sixth aspect, the present invention provides a composition including a bacterial species according to the fourth aspect.

Preferably the composition is in the form of a capsule or tablet or similar formulation however it may also be in the form of a food product.

According to a seventh aspect there is provided a pharmaceutical composition including an effective amount of live bacterial species according to the fourth aspect, together with a pharmaceutically acceptable carrier, adjuvant, solvent or excipient.

Preferably the bacterial species is L. acidophilus.

According to an eighth aspect there is provided a method of assessing efficacy of treatment with live probiotic bacteria or with a composition having live probiotic bacteria, including the steps of:

a measuring the level of salivary immunoglobulin subclass in a sample obtained before commencement of treatment,

b measuring the level of salivary immunoglobulin subclass in a sample obtained after commencement of treatment

c comparing the levels of salivary immunoglobulin subclass in a) and b), wherein the change in immunoglobulin subclass level is indicative of effective treatment.

Preferably the immunoglobulin subclass is IgG1 or IgG2 and it will be understood that equivalent subclasses in various species can also be advantageously used.

According to a ninth aspect there is provided a method of assessing efficacy of treatment with live probiotic bacteria or with a composition having live probiotic bacteria, including the steps of:

a measuring the level of IL-4 or IFN-γ in a sample obtained before commencement of treatment,

b measuring the level of IL-4 or IFN-γ in a sample obtained after commencement of treatment

c comparing the levels of IL-4 or IFN-γ in a) and b), wherein the change in IL-4 or IFN-γ level is indicative of effective treatment.

It will be clear however that any know cytokine marker for the Th1 or the Th2 response will be suitable in place of IFN-γ or IL-4.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Suppression of IgE antibody response in mice fed probiotic bacteria.

Figure 2: Suppression of IgE response to OVA by *L acidophilus* before/after allergen sensitisation

Figure 3: Dose dependency of suppression of IgE response to OVA by *L. acidophilus*

Figure 4: Suppression of IgE response to OVA by live and killed *L acidophilus*

Figure 5: Suppression of IgE response to OVA, and cytokine and IgG antibody subclass response, by *L acidophilus*

DESCRIPTION OF THE PREFERRED EMBODIMENT

A preferred embodiment of the invention will now be described by way of example only.

A mouse animal model was developed which enabled identification of probiotic bacteria capable of downregulating the IgE response to an antigen/allergen. Typically the animal used is a mouse (for example C57BL/6). The animals are fed the candidate bacterial species (alive, dose 10^8-10^{11}, frequency every 1-5 days, for 1-3 weeks). In certain advantageous embodiments the animals are fed the bacteria at the same time as the antigen was being introduced or shortly thereafter, for example the following day. Mice were immunised intraperitoneally with antigen (for example ovalbumin) at an appropriate dose (eg 4-10μg, preferably 8μg), with specific and total IgE measured in serum (eg. at 14 days after immunisation). In certain examples feeding with test bacteria

continues throughout the experiment (see examples). In other examples comparisons are made with killed organisms and dose-response is estimated. In yet other examples the timing of administration of probiotic bacteria in relation to antigen/allergen challenge is examined. The examples demonstrate:

(i) that frequent dosage with live probiotic bacteria at an appropriate does can reduce the IgE response to antigen/allergen.

(ii) that not all probiotic bacteria have the same capacity to reduce the IgE response - thus the model can be used to select candidate bacteria for human use in management or prevention of allergic disease.

(iii) *Lactobacillus acidophilus* is particularly effective in blunting the IgE response to novel antigens.

(iv) that oral administration of the bacterial species affects the systemic IgE antibody response (following injected antigen) and thus may be of value in subjects who have anaphylaxis (eg insect envenomation, and food and drug sensitivities).

(v) probiotic bacteria can downregulate IgE production following antigen administration or exposure to antigen - thus continuous oral ingestion of appropriate doses of live bacteria selected by this method may be of value in the ongoing management of allergic disease.

(vi) administration of live probiotic bacteria is particularly effective

(vii) co-administration of probiotic bacteria with the antigen/allergen, or administration of probiotic bacteria shortly after the antigen/allergen challenge is particularly effective

(viii) to be effective doses of probiotic bacteria in excess of those found in conventional food sources are usually required

The bacterial species identified by the method are useful

(1) for the prevention of allergic disease

(a) in high risk infants (eg those with both parents with allergy)

(b) in those subjected to high risk occupation exposure (eg aluminium smelter workers, woodworkers, chemical factory workers and those working with latex-containing material, especially gloves)

(c) following parenteral exposure to high risk allergens, eg bee venom

(d) in subjects having recognised allergy to specific allergens (eg pre-pollen season, foods, latex, etc)

(2) for the suppression of established allergic disease such as asthma, eczema, hayfever and food allergy.

The probiotic bacteria can be formulated into various compositions and preferably the compositions are pharmaceutical compositions in the form of capsules, tablets, powders and the like. Such formulations can be prepared by known means, using pharmaceutically acceptable carriers, excipients, solvents or adjuvants. Such procedures and ingredients are well know and amply described in standard texts and manuals, for example "Remington: The Science and Practice of Pharmacy", 1995, Mack Publishing Co. Easton, PA 18042, USA, which is incorporated herein by reference.

The probiotic bacteria may also be formulated into food products by the usual well known means.

The invention will now be described more particularly with reference to non-limiting examples.

EXAMPLES

Example 1: Suppression of IgE antibody response to OVA following oral administration of *Lactobacillus.*

Bacteria (*Lactobacillus acidophilus or Lactobacillus casei)* were grown in MRS agar petri dishes (3.8% w/v, Oxoid, Basingstoke, UK). Plates were incubated in 5% CO_2 and air in a humid atmosphere at 37°C for 48 hours.

Bacteria were harvested, washed twice in phosphate buffered saline (PBS) and then resuspended to 3-5 x 10^{10} bacteria per mL in PBS.

Thirty female C57/B16 SPF mice (Animal Resource Centre, Perth, WA, Australia) were fed with 4 or 8 oral doses of 0.6-1.0 x 10^{10} *L acidophilus or L casei* in 0.2 mL PBS or PBS alone given two days apart.

One day after the last oral dose, mice were injected intraperitoneally with 7 μg of egg albumin (ovalbumin, OVA, Sigma-Aldrich, St Loius, Missouri, USA) and 4 mg aluminium hydroxide (Amphojel, Whitehall Laboratories, Sydney, Australia) in 0.2 mL PBS or with PBS alone.

Immunised and control mice were then placed on a feeding regime consisting of 8 oral doses of 0.6-1.0 x 10^{10} bacteria in PBS or PBS alone given two days apart.

Mice were bled by the saphenous vein 2 days after the last dose and the serum collected for IgE antibody determination using an ELISA assay.

The results are shown in Figure 1. Suppression of IgE antibody response to OVA was observed in mice given oral doses of *L acidophilus* and *L casei* compared with immunised mice given PBS. This level of suppression was statistically significant when *L acidophilus* was used.

Example 2: Suppression of IgE response to OVA by *L acidophilus* after allergen sensitisation

C57/Bl6 mice were fed 10^{10} *L acidophilus* before or 24 hrs after sensitisation with 8 μg ovalbumin (OVA) in alum per mouse. Control mice were sham fed with normal saline. In each group, a total of 10 feeds was administered before they were assessed for levels of OVA-specific IgE antibody and total IgE in serum. *L acidophilus* was more effective if administered (or co-administered) at the time of exposure to OVA than when administered prior to allergen exposure, as shown by the suppression of OVA-specific IgE antibody and total IgE responses (Figure 2). Data are mean ± SEM from 5-10 mice. *, $p < 0.05$ compared with control values from saline-fed sensitised mice. Baseline variation reflects different experiments and conditions)

Example 3: Suppression of IgE response to OVA by *L. acidophilus* is dose-dependent

Mice were fed orally with 10^8, 10^9 or 10^{10} *L acidophilus* before they were sensitised 24 hrs later with OVA in alum. Control mice were sham fed PBS. Each dose was administered 10 times every 2 days for 21 days. One week following the final dose, serum IgE and OVA-specific IgE antibody were measured. A dose-dependent suppression of IgE and OVA-specific IgE antibody was noted with a statistically significant effect with 10^{10} bacteria (Figure 3). Data are mean ± SEM from 10 mice. *, $p < 0.05$ compared with control values from saline-fed sensitised mice.

Extrapolation to human subjects indicates the requirement for a dose in the range of approximately 10^{10} to10^{12} live *L acidophilus* each day to effectively downregulate IgE antibody-mediated allergic disease. This is considerably in excess of the numbers of live *Lactobacilli* which can be practically introduced via currently available food products (often about 10^5- 10^7 per serving).

Example 4: Suppression of IgE response to OVA is dependent on live culture of *L acidophilus*

Mice were fed live or formalin-killed 10^{10} *L acidophilus* and then sensitised with OVA as per standard protocol described in Example 3. Figure 4 shows that live bacteria were **more** effective in suppressing IgE response than killed bacteria. Data are mean ± SEM from 5 mice. *, $p < 0.05$ compared with values from saline-fed sensitised mice.

Example 5: Suppression of IgE response to OVA by *L acidophilus* correlates with cytokine and IgG antibody subclass response

Mice fed 10^{10} *L acidophilus* produced higher amounts of IFN-γ and lower amounts of IL-4 in the spleen, a finding consistent with the suppression of IgE response. In addition, the contrasting cytokine patterns correlated with the production of salivary IgG subclass antibodies which showed an upregulation of IgG2a antibody and a downregulation of IgG1, respectively (Figure 5). Data are mean ± SEM from 10 mice. *, $p \leq 0.05$; **, $p < 0.01$ compared with values from saline-fed sensitised mice.

These results indicate that IgG subclass antibody in saliva is an effective surrogate marker for monitoring response of allergic disease to intervention therapy. Thus measurement of equivalent subclasses in humans would be an appropriate laboratory test for effective desensitisation therapy eg. increase in IgG2 anti-allergen antibody and /or decrease in IgG1 antibody in saliva (or other secretions) is indicative of a response to therapy.

Although the invention has been described with reference to specific examples, it will be appreciated by those skilled in the art that the invention may be embodied in many other forms. In particular, it will be clear to the skilled addressee that the methods and compositions described above can be applied to any probiotic which can lower IgE levels in a subject.

CLAIMS:

1. Method of lowering IgE levels by administration of a therapeutically effective amount of live probiotic bacteria, or a live probiotic bacteria-containing composition, to a subject in need thereof.

2. Method of prophylactic or therapeutic treatment of allergy by administration to a subject requiring such treatment a therapeutically effective amount of live probiotic bacteria, or a live probiotic bacteria-containing composition..

3. Method according to claim 1 or claim 2, wherein the probiotic bacteria is, or the probiotic bacteria-containing composition includes, *Lactobacillus*.

4. Method according to any one of claims 1 to 3, wherein the *Lactobacillus* is *Lactobacillus acidophilus* and/or *Lactobacillus casei*.

5. Method according to any one of claims 1 to 4, wherein the probiotic bacteria, or a probiotic bacteria-containing composition is administered at the time of exposure to an antigen/allergen or shortly thereafter.

6. Method according to claim 5, wherein the exposure is natural and/or seasonal exposure or re-exposure to antigen/allergen.

7. Method according to claim 5, wherein the exposure is artificial exposure to antigen/allergen.

8. Method according to claim 7, wherein the artificial exposure is the injection of antigen/allergen.

9. Method according to claim 7, wherein the artificial exposure is under-tongue administration of antigen/allergen.

10. Method according to any one of claims 1 to 9, wherein the amount of probiotic bacteria administered is at least 10^{10} live bacteria.

11. Method according to claim 10, wherein the amount of probiotic bacteria administered is from about 10^{10} to about 10^{12} bacteria.

12. Method according to any one of claims 1 to 11, wherein the probiotic bacteria or the probiotic-bacteria containing composition is administered daily.

13. Method according to any one of claims 1 to 12, wherein the probiotic bacteria, or the probiotic bacteria-containing composition, is in a solid dosage form.

14. Method according to claim 13, wherein the probiotic bacteria, or the probiotic bacteria-containing composition, is in tablet or capsule form.

15. Method according to any one of claims 1 to 12, wherein the probiotic bacteria, or the probiotic bacteria-containing composition, is in the form of a food product.

16. Method according to any one of claims 1 to 15, wherein the subject has recognised allergic disease to specific allergens and wherein the specific allergens are present in the pollen season, foods or latex-containing materials.

17. Method according to any one of claims 1 to 16, wherein the subject is prone to anaphylaxis and wherein the trigger for the allergic response is insect envenomation, food and/or drug sensitivities.

18. Method according to any one of claims 1 to 17, wherein the subject is exposed to an allergen parenterally.

19. Use of live probiotic bacteria for the manufacture of a medicament for lowering IgE levels.

20. Use of live probiotic bacteria for the manufacture of a medicament for treating allergy.

21. Method of identifying a bacterial species capable of lowering IgE levels in a mammal including:

a) administration of the bacterial species to a mammal;

b) administration of an allergen (antigen) to the mammal; and

c) determination of the IgE antibody and/or total IgE level in the mammal after treatment with the bacterial species and comparison with a control mammal in which a bacterial species was not administered,

wherein steps (a) and (b) can be performed consecutively in any order or simultaneously.

22. Method according to claim 21, wherein the bacterial species is a *Lactobacillus* species.

23. Method according to claim 22, where the bacterial species is *Lactobacillus acidophilus*.

24. Method according to any one of claims 21 to 23, wherein 1 to 20 oral doses of bacteria are administered prior to administration of the antigen.

25. Method according to claim 24, wherein 4 to 8 oral doses of bacteria are administered.

26. Method according to any one of claims 21 to 25, wherein administration of the oral doses of bacteria is at 1 to 5 day intervals.

27. Method according to claim 26, wherein administration of the oral doses of bacteria is at 2 day intervals.

28. Method according to any one of claims 21 to 27, wherein the administration is over a 1 to 3 week period.

29. Method according to claim 28, wherein the administration is over a 1 to 2 week period.

30. Method according to any one of claims 21 to 29, wherein the bacterial species is administered in the amount of about 10^8 to about 10^{11} live bacteria.

31.. Method according to any one of claims 21 to 30, wherein the antigen is administered at a dose of 4 to 10 μg.

32. Method according to claim 31, wherein the antigen is administered at a dose of 8 μg.

33. Method according to any one of claims 21 to 32, wherein the antigen is administered intraperitoneally.

34. Method according to any one of claims 21 to 33, wherein the antigen is administered at the same time or shortly after administration of the bacterial species.

35. Method according to any one of claims 21 to 34, wherein the antigen is administered 1 day after the administration of 4 to 8 oral doses of bacteria.

36. Method according to any one of claims 21 to 35, wherein administration of the bacteria species is continued after administration of the antigen.

37. Method according to any one of claims 21 to 36, wherein about 8 oral doses of bacteria are administered.

38 Method according to any one of claims 21 to 37, wherein the bacteria are administered at 2 day intervals.

39. Method according to any one of claims 21 to 38, wherein IgE is measured in serum and wherein the serum is collected approximately 14 days after administration of the antigen.

40. Method according to any one of claims 21 to 39, wherein IgE antibody level is determined using an immunoassay.

41. Bacterial species identified by the method according to any one of claims 21 to 40.

42. Composition including an effective amount of live bacterial species according to claim 41.

43. Composition according to claim 42 in form of a tablet or capsule.

44. Composition according to claim 42 in form of a food product

45. Pharmaceutical composition including an effective amount of live bacterial species according to claim 41, together with a pharmaceutically acceptable carrier, adjuvant, solvent or excipient.

46. Bacterial species according to claim 41, which is L. acidophilus.

47. Composition according to any one of claims 42 to 45, wherein the bacterial species is L. acidophilus.

48. Method according to any one of claims 2 to 18, wherein the allergy is selected from the group consisting of asthma, eczema, hayfever and food allergy.

49. Method of assessing efficacy of treatment with live probiotic bacteria or with a composition having live probiotic bacteria, including the steps of:

a measuring the level of salivary immunoglobulin subclass in a sample obtained before commencement of treatment,

b measuring the level of salivary immunoglobulin subclass in a sample obtained after commencement of treatment

c comparing the levels of salivary immunoglobulin subclass in a) and b), wherein the change in immunoglobulin subclass level is indicative of effective treatment.

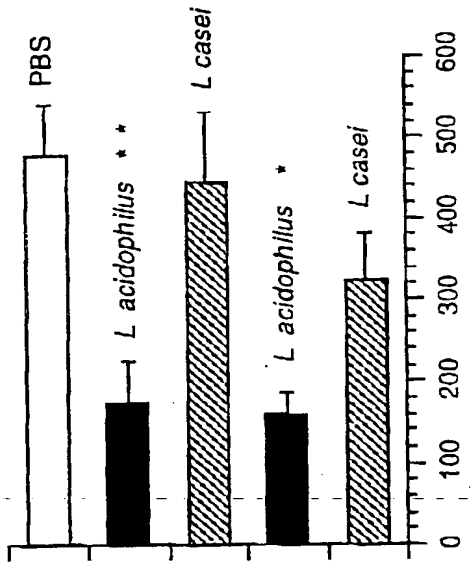
50. Method according to claim 49, wherein the immunoglobulin subclass is IgG1 or IgG2.

51. Method of assessing efficacy of treatment with live probiotic bacteria or with a composition having live probiotic bacteria, including the steps of:

a measuring the level of IL-4 or IFN-γ in a sample obtained before commencement of treatment,

b measuring the level of IL-4 or IFN-γ in a sample obtained after commencement of treatment

c comparing the levels of IL-4 or IFN-γ in a) and b), wherein the change in IL-4 or IFN-γ level is indicative of effective treatment.


PBS
L acidophilus * *
L casei
L acidophilus *
L casei
0
100
200
300
400
500
600
OVA IgE Ab (ng/mL)
* * p < 0.01
* p < 0.03
Oral Doses
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Post-immunisation
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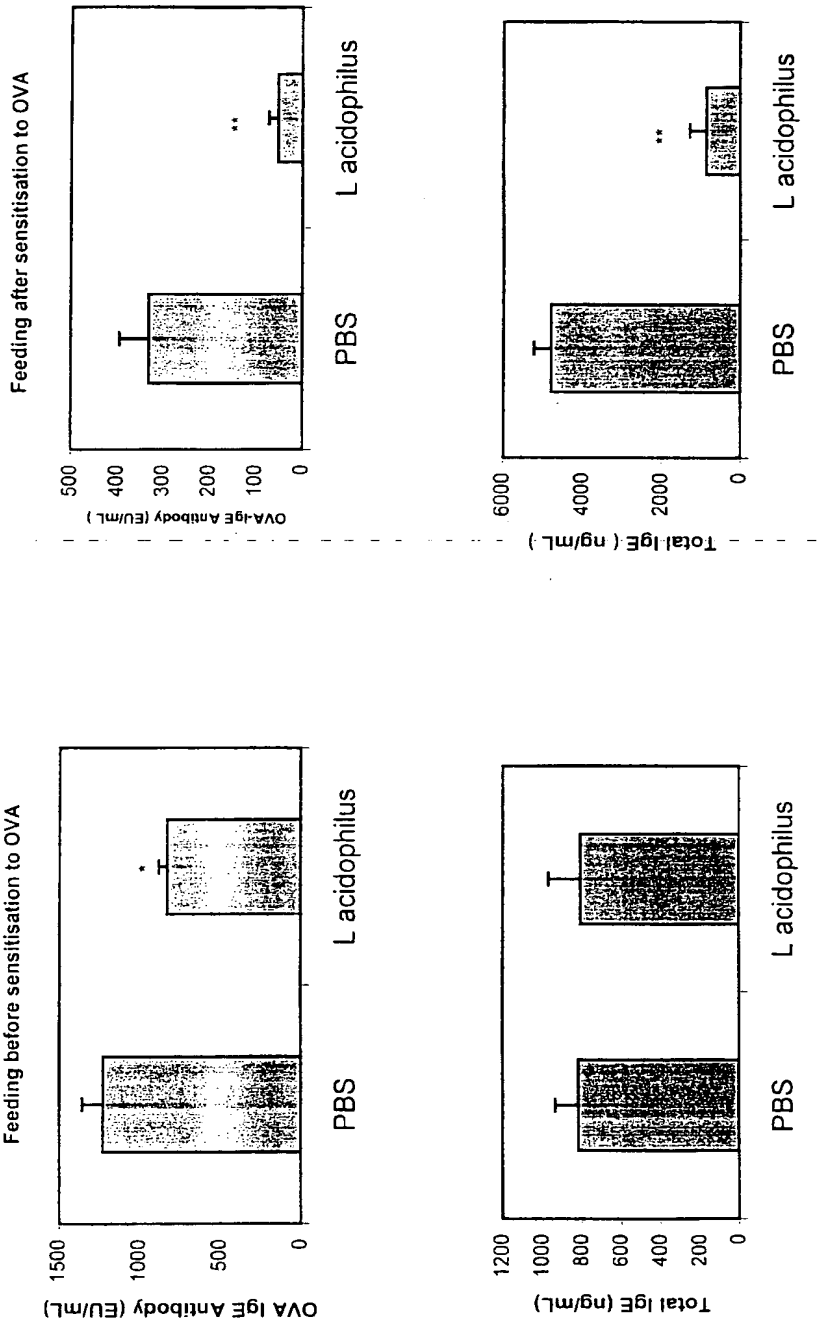
4X
4X
8X
8X
8X
8X
8X
8X

Fig. 1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300
200
100
0
**
PBS
L acidophilus
Total IgE (ng/mL)
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4000
2000
0
**
PBS
L acidophilus
Feeding before sensitisation to OVA
OVA IgE Antibody (EU/mL)
1500
1000
500
0
*
PBS
L acidophilus
Total IgE (ng/mL)
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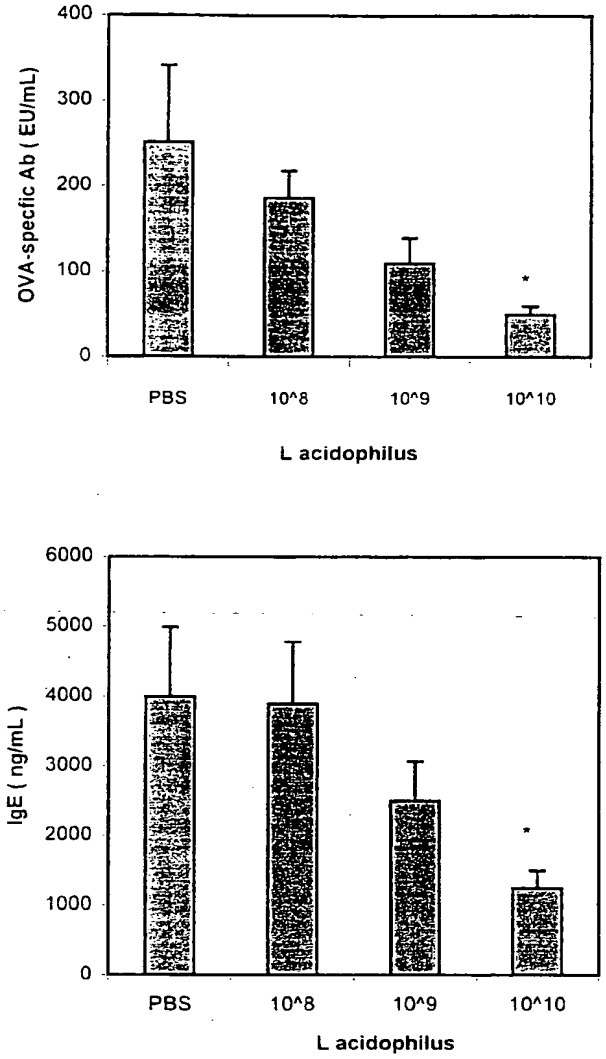
1000
800
600
400
200
0
PBS
L acidophilus

Fig. 2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300
200
100
0
OVA-specific Ab (EU/mL)
*
PBS
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10^9
10^10
L acidophilus
6000
5000
4000
3000
2000
1000
0
IgE (ng/mL)
*
PBS
10^8
10^9
10^10
L acidophilus

Fig. 3

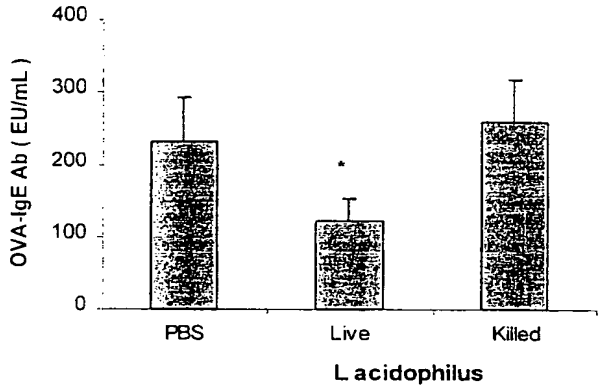
400
300
200
100
0
OVA-IgE Ab (EU/mL)
*
PBS
Live
Killed
L acidophilus

Fig. 4

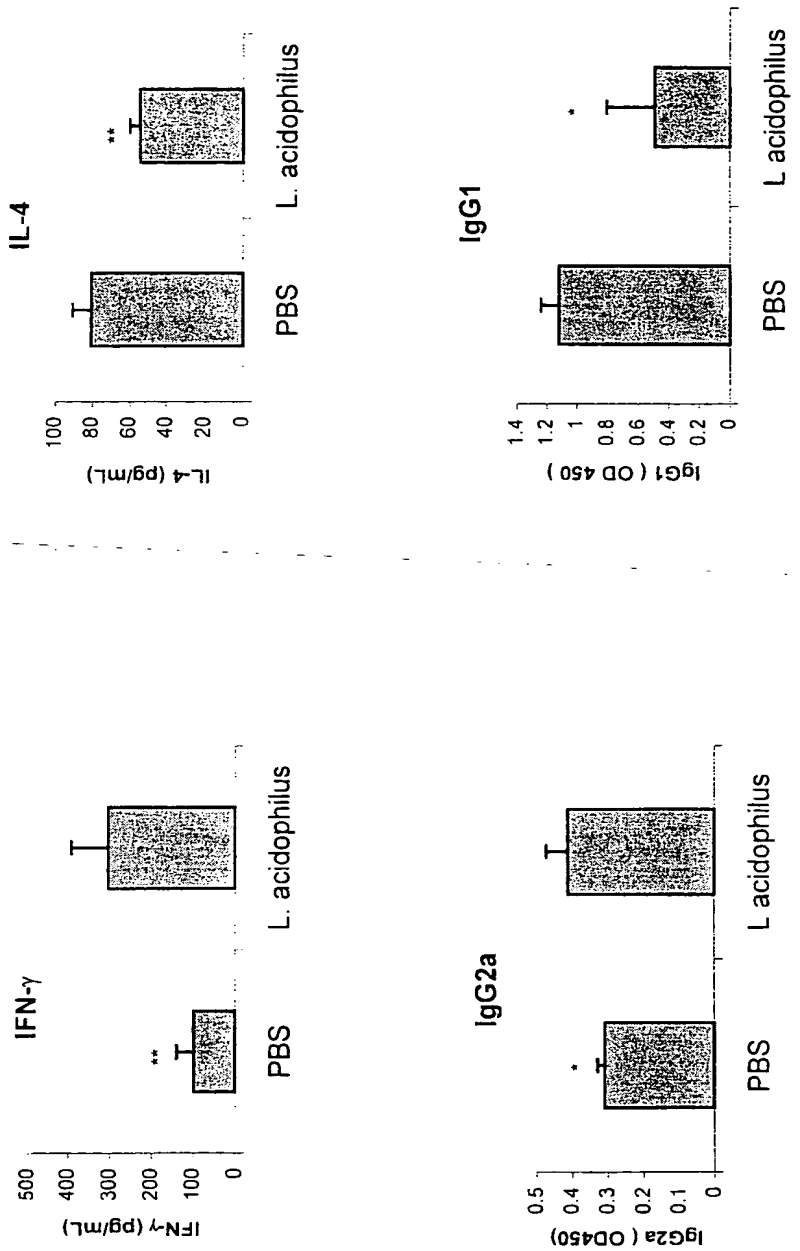
IL-4
IL-4 (pg/mL)
100
80
60
40
20
0
**
PBS
L. acidophilus
IgG1
IgG1 (OD 450)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
PBS
L acidophilus
IFN-γ
IFN-γ (pg/mL)
500
400
300
200
100
0
**
PBS
L. acidophilus
IgG2a
IgG2a (OD450)
0.5
0.4
0.3
0.2
0.1
0
*
PBS
L acidophilus

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01414

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 39/07; A61P 37/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
DERWENT DATABASE - WPAT; CHEMICAL ABSTRACTS

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
MEDLINE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT; CHEMICAL ABSTRACTS; MEDLINE- Keywords in Supplemental Box

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/42568 A (MENDES S.R.L.) 26 August 1999 (see pages 5-7, examples and claims)	1-51
X	Patent Abstracts of Japan, JP 11 199495 A (NICHINICHI SEIKAYU, KK MORISHITA JINTANCO LTD) 27 July 1999 (see entire abstract)	1-51
X	EP 904 784 A (N.V. NUTRICIA) 31 March 1999 (see entire document)	1-51

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
17 January 2001

Date of mailing of the international search report
25 January 2001

Name and mailing address of the ISA/AU

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01414

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU 61278/96 (710126) B opi. 15 January 1997 (see entire document)	1-51
X	Patent Abstracts of Japan, JP 09 002959 A (YAKULT HONSHA CO LTD.) 7 January 1997 (see entire abstract)	1-51
X	ANTONIE VAN LEEUWENHOEK, 1999, 76(1-4), "Immunomodulatory function of lactic acid bacteria", Yasui H. et. al., pages 383-389 (see entire document)	1-51
X	INTERNATIONAL ARCHIVES OF ALLERGY & IMMUNOLOGY, 1999, 115 "*Lactobacillus casei* inhibits antigen-induced IgE secretion through regulation of cytokine production in murine splenocyte cultures", Shida K. et.al., pages 278-287 (see entire document)	1-51
X	J DAIRY SCIENCE, 1998, 81, "The effect of oral feeding of *Lactobacillus casei* strain Shirota on immunoglobulin E production in mice", Matsuzaki T. et.al., pages 48-53 (see entire document)	1-51
A	TRENDS IMMUNOLOGY TODAY, September 1999, 20(9), "Immunity and probiotics", Dugas B. e.al., pages 387-390 (see entire document)	1-51
P,X	JP 2000 004830 (ASAMA KASEI KK et.al.) (Abstract only) 1 November 2000 (see entire abstract)	1-51
P,X	J OF THE AMERICAN COLLEGE OF NUTRITION, 2000, 19(2), "Effect of lactic acid bacteria on diarrheal diseases", Heyman M., pages 137S-146S (see entire document)	1-51

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01414

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: B

WPAT Keywords-

(probiot+ or microb+ or bacteri+ or +bacil+) and (immunoglobulin# or ig+) and (allergy or hypersensitiv+ or anaphyl+);

(probiot+ or microb+ or bacteri+ or +bacil+) and (ige or immunoglobulin e);

(probiot+ or microb+ or bacteri+ or +bacil+) and (allergy or hypersensitiv+ or anaphyl+)

CHEMICAL ABSTRACTS Keywords-

[(probiotics/IT or supplements/IT) or (probiotic)] and (dietary supplements/IT or food supplements/IT or hypersensitivity/IT or immunoglobulins/IT or ige/IT or immediate hypersensitivity/IT

MEDLINE Keywords-

(probiotics/CT or supplement/CT or supplement, dietary/CT or dietary supplement/CT) and (hypersensitivity/CT or immunoglobulin/CT or immunoglobulin e/CT or immnuoglobulins/CT);

(probiotics/CT or supplement/CT or supplement, dietary/CT or dietary supplement/CT) and (immunoglobulins e/CT or immediate hypersensitivity/CT or hypersensitivity, immediate/CT or ige/CT)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/01414

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	99/42568	AU	96441/98	BR	9815677	EP	1058725
		IT	1298918	ZA	9811619		
JP	11 199495						
EP	904 784						
AU	61278/96	WO	97/00078	BG	9607827	EP	833649
		JP	11 510791	NO	975832	SK	1715/97
		CA	2224320	CN	1187772	CZ	9703997
		FI	104465	IL	122577	PL	324010
		ZA	9605088				
JP	09 002959						

END OF ANNEX